Adipose tissue angiogenesis

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ABSTRACT: A review of adipose tissue angiogenesis includes the morphological and cytochemical development of adipose tissue vasculature and the concept of primitive fat organs. Spatial and temporal relationships between fetal vascular and fat cell development are discussed, including depot- and genetic-dependent arteriolar differentiation. The relationship between connective tissue deposition and elaboration of adipose tissue vasculature is discussed with respect to regulating adipocyte development in a depot-dependent manner. In vitro studies indicated that depot-dependent vascular traits may be attributable to intrinsic growth characteristics of adipose tissue endothelial cells. These studies indicate that adipogenesis may be regulated by factors that drive angiogenesis. Fundamental aspects of angiogenesis, including basement membrane breakdown, vasculogenesis, angiogenic remodeling, vessel stabilization, and vascular permeability were reviewed. Critical angiogenic factors include vascular endothelial growth factor (VEGF), VEGF receptors, angiopoietins (Ang), ephrins, matrix metalloproteinases, and the plasminogen enzymatic system. Vascular endothelial growth factor is the most critical factor because it initiates the formation of immature vessels and disruption of a single VEGF allele leads to embryonic lethality in mice. Expression of VEGF is influenced by hypoxia, insulin, growth factors, and several cytokines. Angiogenic factors secreted and/or produced by adipocytes or preadipocytes are discussed. Vascular endothelial growth factor expression and secretion by adipocytes is regulated by insulin and hypoxia, and is associated with adipose tissue accretion. Vascular endothelial growth factor accounts for most of the angiogenic activity of adipose tissue. The proposed role of leptin as an adipogenic factor is reviewed with respect to efficacy on various aspects of angiogenesis relative to other angiogenic factors. The VEGF and leptin genes are both hypoxia inducible, but potential links between VEGF and leptin gene expression have not been examined. Finally, several studies including a study of mice treated with antiangiogenic factors indicate that adipose tissue accretion can be controlled through the vasculature per se.

Key Words: Adipose Tissue Development, Blood Vessel Development, Capillaries, Leptin, Vascular Endothelial Growth Factor

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Introduction

The discovery of the ob, or leptin, gene has provided a new perspective on adipose tissue function because it is the primary source of circulating leptin (review: Ahima and Flier, 2000). Considering the actions of leptin on growth, metabolism, behavior, and the numerous other secretory factors produced by adipose tissue (Ahima and Flier, 2000), it can now be considered an endocrine organ. In particular, very high levels of IGF-I produced by fetal pig adipose tissue may indicate an endocrine function of adipose tissue IGF-I. The development and maturation of adipose tissue vascularity (Crandall et al., 1997) are critical to the function of adipose tissue as an endocrine organ, but adipose tissue angiogenesis is poorly understood. Therefore, it is critical to review the factors that regulate adipose tissue angiogenesis.

Development of Adipose Tissue Vasculature

Fat cell development is characterized by the appearance of a number of fat cell clusters, or "primitive organs," which subsequently increase in number and size throughout fetal development (Table 1; reviews: Hausman et al., 1990; Hausman and Hausman, 2003; Crandall et al., 1997; Martin et al., 1998). Primitive fat organs are vascular structures in presumptive adipose tissue with few or no fat cells and are observed in fetal sheep, cattle, and pigs (Figure 1). Fetal adipocyte development is spatially and temporally related to capillary

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Table 1. Evidence of autocrine/paracrine or developmental relationships between capillaries/endothelial cells and preadipocytes[a]

Studies/experiments	Results/observations	Reference
Electron microscopic studies in vivo	Capillary differentiation concurrent with early stages of adipogenesis in perivascular preadipocytes	Hausman and Richardson (1982); Iyama et al. (1979)
Human preadipocytes and endothelial cells in vitro	Migration regulated by PAI-1 and both express and secrete PAI-1 and express the $\alpha_v\beta_3$ integrin	Crandall et al. (2000)
Cell replication in vitro	Depot dependent for preadipocytes and endothelial cells in rats	Lau et al. (1990; 1996)
Adipocyte conditioned media	Hypoxia enhanced VEGF secretion	Mick et al. (2002); Zhang et al. (1997)
Adipocyte and lung endothelial cell co-cultures	Increased preadipocyte replication and development in vitro—not soluble factors	Aoki et al. (2003)
AD-3/AD-1 immunostaining in developing adipose tissue	Reactive preadipocytes and capillary endothelial cells	Wright and Hausman (1990a,b)

[a]PAI-1 = plasminogen activator inhibitor 1; VEGF = vascular endothelial growth factor; AD-3/AD-1 = monoclonal antibody markers of porcine preadipocytes/adipocytes.

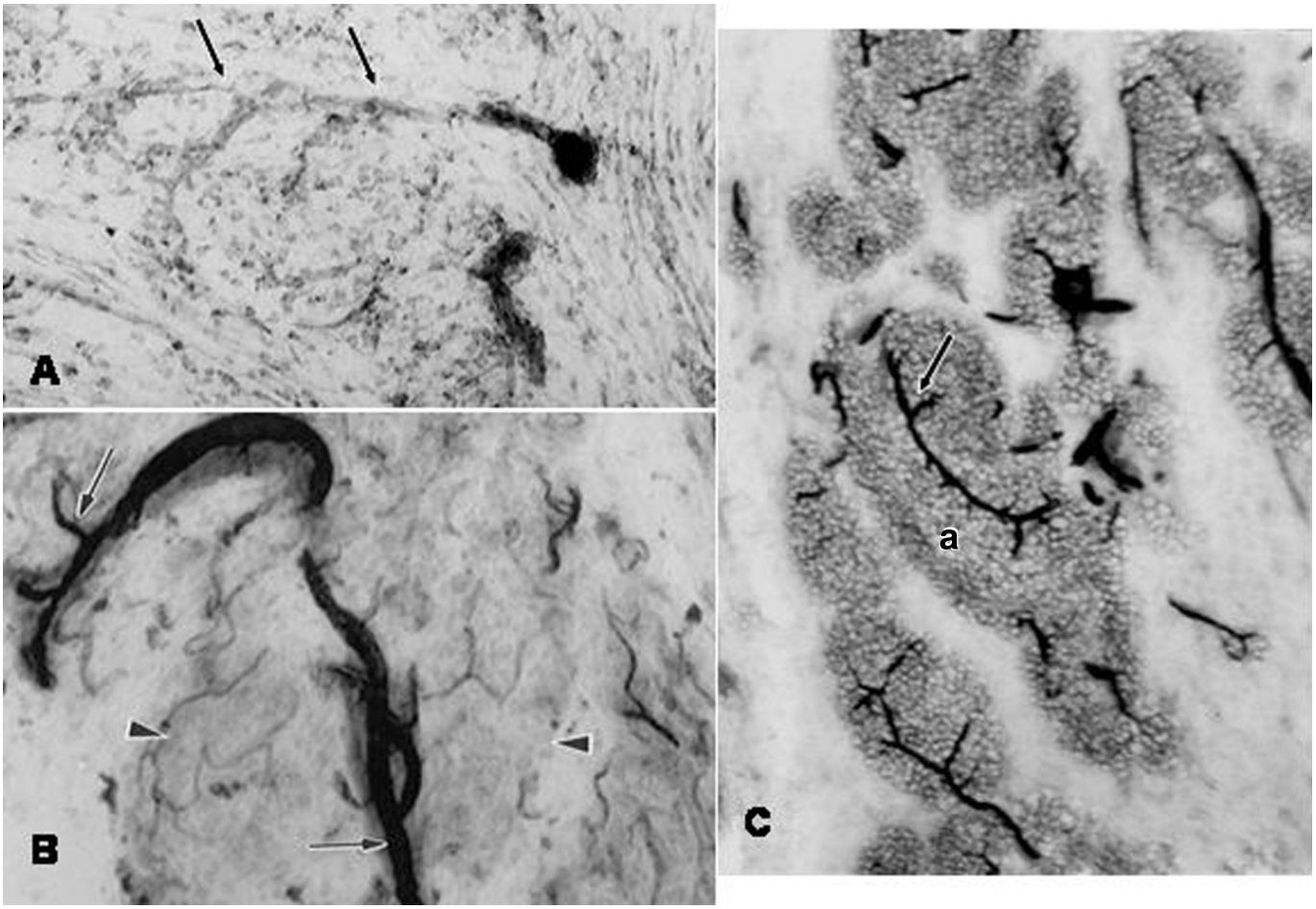

Figure 1. Phosphatase histochemistry in cryostat sections of fetal perirenal adipose tissue from A) 70-, B) 90-, and C) 105-d fetal pigs. Note that phosphatase reactivity is limited in arterioles (arrows) in perirenal tissue at 70 d (A), whereas more extensive phosphatase reactivity indicates that arteriolar (arrows) differentiation has clearly progressed by 90 (B) and 105 (C) d. Arteriolar (arrows) differentiation precedes adipocyte (a) development because there are virtually no adipocytes associated with differentiating arterioles (arrows) at d 90 (B). Areas within perirenal tissue at 90 d (B, arrowheads) can be considered primitive fat organs because there are few to no fat cells but they are morphologically similar to adipocyte- (a) filled areas of adipose tissue at 105 d (C) (×300).

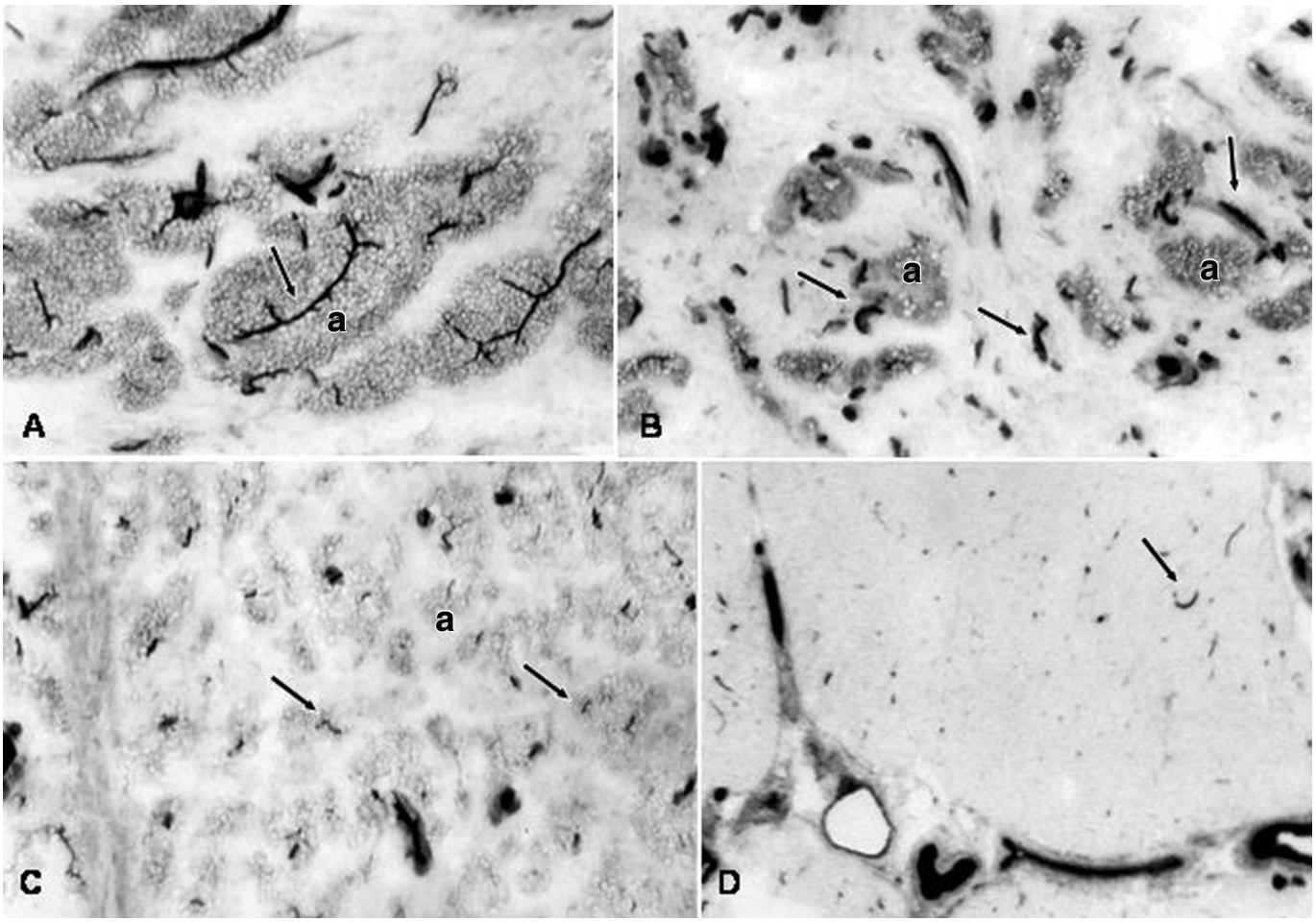

Figure 2. A, B) Phosphatase histochemistry in cryostat sections of perirenal and C) middle subcutaneous adipose tissue and the red or deep portion of the D) semitendinosus muscle from 105-d pig fetuses. Note that phosphatase reactivity clearly delineates arteries and arterioles (arrows) in adipose and muscle tissue. Arteriolar development is similar in perirenal tissues (A, B) regardless of the extent of adipocyte cluster (a) development. Arterioles (arrows) associated with fat cell clusters (a) are larger in perirenal (A, B) adipose tissues than in subcutaneous (C) adipose tissues. Remarkably, arterioles are more numerous and larger in adipose tissue than in muscle (D) (×300).

development (Crandall et al., 1977). Arteriolar development clearly precedes adipocyte differentiation in internal fat depots and can be used to distinguish adipose tissue depots in the fetus (Figure 2; Crandall et al., 1997). The degree of overlap between fat cell and vascular morphological development is depot dependent, whereas fat cell size is independent of depot. Vascular development may be intrinsic to the fat depot because Lau and co-workers showed that endothelial cells derived from several rat fat depots had depot-dependent replication rates in cell culture after several passages (Table 1; Lau et al., 1990, 1996). Although angiogenesis appears to be linked to adipogenesis, the collective reports of genes and proteins expressed by meat animal adipose tissue indicates no reports of gene or protein expression of the major regulators of angiogenesis (Hausman and Hausman, 2003).

Developmental Relationships Between Adipogenesis and Angiogenesis

There is multifaceted evidence of autocrine/paracrine or developmental relationships between capillaries/endothelial cells and preadipocytes (Table 1). Recent studies show that human preadipocytes and endothelial cells express the $\alpha_v\beta_3$ integrin and express and secrete PAI-1, which regulates preadipocyte and endothelial cell migration in vitro (Crandall et al., 2000). These findings provide a mechanism for the formation of primitive fat organs because preadipocytes would migrate with developing capillary endothelial cells during angiogenesis (Crandall et al., 2000). Furthermore, the production and secretion of PAI-1 by preadipocytes would ensure coordination of adipogenesis and angiogenesis at the local level (Crandall et al., 2000).

Connective Tissue Deposition, Adipogenesis, and Angiogenesis

Connective tissue deposition and blood vessel development or elaboration is developmentally linked in adipose tissue. Phosphatase cytochemical studies of fetal pig adipose tissue show that capillary beds in dense connective tissue are immature and contain few adipocytes, whereas capillary beds in loose connective tissue are mature and contain many more adipocytes (review: Crandall et al, 1997; Hausman and Thomas, 1984). Experimentally induced and age-associated antagonism of dense connective tissue development results in development of mature capillary beds and enhanced fat cell development (Hausman and Thomas, 1984). Dense connective tissue is evident in late-term fetal adipose depots but is virtually absent in adipose depots in young and growing pigs (Hausman and Kauffman, 1986). Therefore, as dense connective tissue breaks down immature adipose tissue capillaries may elaborate and mature, potentiating or stimulating adipocyte development.

Extracellular Proteolysis and Angiogenesis

A critical aspect of angiogenesis is the breakdown and degradation of connective tissue and basement membrane proteins (Figure 3; Yancopoulos et al., 2000). For instance, angiogenic sprouting is dependent on basement membrane protein degradation (Figure 3). Extracellular proteolysis of these proteins involves two families of proteolytic enzymes, the plasminogen (**Plg**) activator (**PA**)-plasmin system and the matrix metalloproteinases (reviews: Pepper, 2001; Papetti and Herman, 2002; Liekens et al., 2001). Extracellular proteolysis is implicated in many processes, including basement membrane degradation, cell migration/ECM invasion, capillary lumen formation, regulation of cytokine activity, and release of membrane bound vascular endothelial growth factor (**VEGF**) and tumor necrosis factor-α (**TNF**α; Pepper, 2001). Inhibitors of matrix metalloproteinase (**MMP**) activity and knockout of either MMP-2, MMP- 9, or membrane-type inhibitor-MMP genes either blocks or reduces angiogenesis, whereas deficiencies in either urokinase-type PA (**u-PA**), uPA receptor, tissue-type PA (**t-PA**) or Plg genes has no apparent influence on developmental angiogenesis (Pepper, 2001).

Adipose Tissue PA-Plasmin System and Matrix Metalloproteinases

Plasminogen activator inhibitor-1 (**PAI-1**) inhibits t-PA and u-PA and influences migration of endothelial, smooth muscle cells and human preadipocytes and hypoxia induces PAI-1 expression in microvascular endothelial cells (Pepper, 2001). Plasminogen activator inhibitor-1 is one of the most studied factors secreted by adipose tissue because of the adverse health consequences of elevated circulating PAI-1 levels. Plasminogen activator inhibitor-1 expression and secretion by human fat is depot dependent and stromal-vascular cells are the primary source of PAI -1 expression and secretion (Bastelica et al., 2002). Troglitazone (**TGZ**) reduces human fat PAI-1 secretion, which is associated with improved endothelial function in chronic disease states (Bastelica et al., 2002; Gottschling-Zeller et al., 2000; Paradisi et al., 2003).

Plasminogen activator inhibitor-1 gene knockout in mice increased t-PA levels and decreased adipose tissue endothelial cells but did not influence adipose tissue development (Morange et al., 2000). Changes in adipose tissue endothelial cells or capillaries were not associated with decreased adipose tissue development and body weights in mice that overexpress PAI-1 (Lijnen et al., 2003). Related studies showed that fat tissue weights and fat tissue capillaries were decreased in Plg knockout mice, whereas fat tissue weights, fat cell size, and fat tissue endothelial cell number were increased in t-PA knockout mice (Hoover-Plow et al., 2002; Morange et al., 2000, 2002) . Despite the apparent contradictory nature of these results, these studies do indicate a role for the PA-plasmin system in adipose tissue angiogenesis.

Many matrix metalloproteinases (MMP), including MMP-2, MMP-9, and their tissue inhibitors (**TIMP**), are expressed by adipose tissue and stromal-vascular cells in a depot-dependent manner (Maquoi et al., 2002; Bouloumie et al., 2001; Chavey et al., 2003). Matrix metalloproteinase inhibitors, MMP, and TIMP gene deficiencies (knockouts) markedly influence adipose tissue development but may do so in conjunction with or even independent of angiogenesis (Croissandeau et al., 2002; Lijnen et al., 2002a,b). For instance, matrix metalloproteinases and their inhibitors influence overall extracellular matrix degradation and remodeling during adipose tissue expansion (Chavey et al., 2003; Lijnen et al., 2002a,b, 2003). Recent studies of 3T3-L1 preadipocytes also indicate that matrix metalloproteinases may mediate adipocyte differentiation independent of angiogenesis (Croissandeau et al., 2002; Bouloumie et al., 2001; Chavey et al., 2003).

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is the only true endothelial cell growth factor and is necessary to initiate the formation of immature vessels by either vasculogenesis or angiogenic sprouting in the adult and during development (Figure 3; reviews: Papetti and Herman, 2002; Liekens et al., 2001; Yancopoulos et al., 2000; Ribatti et al., 2003). Of six isoforms transcribed from a single gene, $VEGF_{165}$ is the most commonly expressed (Yancopoulos et al., 2000). Vascular endothelial growth factor with the VEGF receptors VEGFR-1 and VEGFR-2 influence endothelial cell proliferation, migration and survival, tubulogenesis, vascular permeability, the MMP proteinases, and the PA system. Ei-

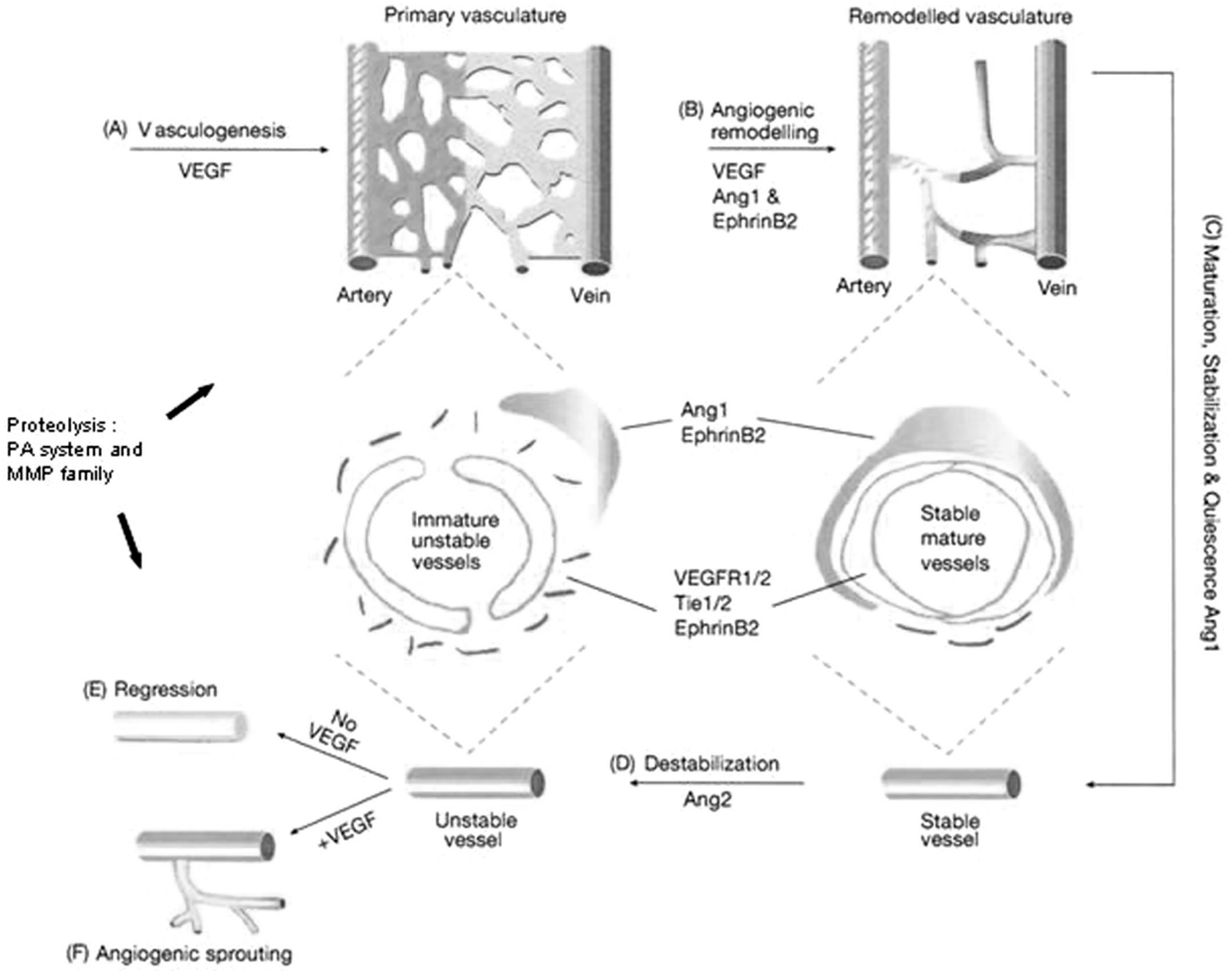

Figure 3. Schematic representation of the roles of vascular endothelial cell growth factor (VEGF) and angiopoietin (Ang)-1 and Ang-2 during blood vessel formation. MMP = matrix metalloproteinases; PA = plasminogen activator; VEGFR = VEGF receptor. Used with permission by the Nature Publishing Group, from Yancopoulos et al., Nature 407:242, 2000 (http://www.nature.com).

ther loss of a single VEGF allele or deficiency of VEGFR-1 or VEGFR-2 results in embryonic lethality. Mice deficient in VEGF and VEGFR-2 are also virtually devoid of vascular structures, but vascular structures are evident in VEGFR-1 deficient mice but impaired assembly of vessels indicates a role for VEGFR-1 in vascular remodeling as opposed to vasculogenesis. Vascular endothelial growth factor gene expression is influenced by a number of factors, including hypoxia, insulin, growth factors, and several cytokines (Liekens et al., 2001). Hypoxia-induced VEGF gene transcription and RNA stability is mediated through the binding of hypoxia-inducible factor (**HIF**) to *cis* elements in the VEGF promoter (Liekens et al., 2001).

Adipose Tissue Vascular Endothelial Growth Factor

Adipose tissue VEGF has not been studied in meat animals but has been examined to some extent in the rat and rabbit (Mick et al., 2002; Zhang et al., 1997; Morimura et al., 2001; Asano et al., 1997, 1999, 2001). Vascular endothelial growth factor accounts for the major portion of the angiogenic activity of media conditioned by adipocytes and adipose tissue per se (Zhang et al., 1997). Most of adipose tissue VEGF expression and secretion is attributable to adipocytes in the rat and mouse (Mick et al., 2002; Zhang et al., 1997; Ross et al., 2002). Although not normalized for adipocyte number, VEGF protein expression and secretion in the rat may be depot dependent and highest in the omental depot (Zhang et al., 1997). Adipose tissue VEGF expression and secretion in rats is induced by hypoxia and insulin but is not influenced by glucocorticoids or leptin (Mick et al., 2002; Zhang et al., 1997). Insulin may be involved in increased adipose tissue VEGF expression during rebound weight gain after diet restriction in rabbits (Morimura et al., 2001). There are no other studies of nutritional or hormonal regulation of adipose

tissue VEGF production, and VEGF receptors have not been examined in adipose tissue.

Several studies have examined the regulation of brown adipose tissue (**BAT**) VEGF expression in the rat (Asano et al., 1997, 1999). Vascular endothelial growth factor expression is much greater in BAT than in white adipose tissue (**WAT**; Asano et al., 1997, 1999). Exposure to cold temperatures rapidly and transiently induces BAT VEGF gene expression, which is maximized after just 1 h of cold exposure (Asano et al., 1997, 1999). Further studies showed that the adrenergic system mediates the cold-induced VEGF expression in BAT possibly through the β-adrenergic pathway (Asano et al., 1999).

Angiopoietin (**Ang**)-1, Ang-2, and Tie 2 and Tie 1 receptors are considered VEGF's most important partners (Figure 3); Yancopoulos et al., 2000; Liekens et al., 2001; Papetti and Herman, 2002). Tie 2 is an Ang receptor, whereas the ligand for Tie 1 is unkown. The Tie 1 and 2 and VEGF-1 and - 2 receptors have similar developmental expression patterns (Yancopoulos et al., 2000). Studies of gene knockout and overexpression in mice have provided considerable insight into the function of the Ang system (Papetti and Herman, 2002; Liekens et al., 2001; Yancopoulos et al., 2000). Tie 1-deficient mice die embryonically, and the vascular integrity of developing blood vessels is compromised. A deficiency in either Ang-1 or Tie 2 results in a normal primary vasculature, but impaired vascular remodeling is observed, indicating a role in sprouting and branching of vessels in angiogenesis. Consistent with these observations, Ang-1 overexpression results in more vessels that are more highly branched and larger in diameter than in wild-type mice. Overexpression of Ang-2 results in lethality and vascular defects similar to those seen in Ang-1 and Tie 1 knockout mice. Therefore, this and other evidence indicate that Ang-1 stabilizes blood vessel walls, whereas Ang-2 destabilizes blood vessels by antagonizing Ang-1 in blood vessels (Figure 3). Angiopoietin-2 may check or prevent excessive branching and sprouting by destabilizing blood vessels (Papetti and Herman, 2002; Liekens et al., 2001; Yancopoulos et al., 2000). In microvascular endothelial cells, hypoxia, VEGF, FGF, and cytokines induced Ang-2 gene expression, whereas Ang-1 expression was not influenced by VEGF and hypoxia (Oh et al., 1999; Mandriota and Pepper, 1998). Except for a study of Ang-2 gene expression in preadipocytes and ob/ob fat tissue (Cohen et al., 2001), there are virtually no studies of adipose tissue angiopoietins.

Ephrin-B2/EphB4

Ephrin-B2 and its receptor, EphB4, are involved in establishing the arterial vs. venous identity, that is, defining the boundaries of arteriovenous domains (Yancopoulos et al., 2000; Liekens et al., 2001; Papetti and Herman, 2002; Hamada et al., 2003). In contrast to VEGF and Ang, ephrins (Figure 3) must be membrane bound to activate their receptors. Eph/ephrin signaling is mediated by cell-to-cell interaction, resulting in repulsive or attractive signaling. Mice lacking both genes are defective in early angiogenic modeling. Aberrant vessel projection and vascular network formation are observed in transgenic mice that constitutively and ubiquitously express ephrin-B2, whereas these abnormalities are not observed in mice overexpressing ephrin-B2 only in vascular endothelial cells (Oike et al., 2002).

Integrin $\alpha_v\beta_3$

Other key membrane bound factors include the integrin $\alpha_v\beta_3$, which is critical for vasculogenesis and angiogenesis during development (Papetti and Herman, 2002; Liekens et al., 2001). It is highly expressed on activated endothelial cells and mediates endothelial cell attachment, spreading, and migration. Integrin $\alpha_v\beta_3$ binds MMP-2 and localizes the active form of the enzyme to the tips of angiogenic vessels. It binds vitronectin, fibronectin, and fibrin. Knockout of either α_v or β_3 gene results in excessive bleeding but normal blood vessel development.

Growth Factors/Cytokines

Gene knockout studies in mice have demonstrated key roles for growth factors during angiogenesis (Papetti and Herman, 2002). In mice deficient in either platelet-derived growth factor (**PDGF**)-B or PDGF-β, blood vessels appear normal but mice hemorrhage and are edematous because of a lack of pericyte proliferation and migration along angiogenic sprouts. These and other studies indicate that PDGF recruits pericytes to preformed capillaries or induces proliferation of pericytes already recruited. Regardless, the actions of PDGF help maintain capillary wall stability.

Mice defective in either transforming growth factor (TGF)-β1or TGF-β receptor 1 have frail and incomplete blood vessel walls, indicating a role in establishing the integrity of vessel sprout walls (Papetti and Herman, 2002). These and other studies demonstrate that TGF-β1 establishes a scaffold that favors formation of vessel tubes by modulating the synthesis of extracellular matrix (**ECM**) components, proteases, and their inhibitors. Transforming growth factor-β1 also establishes and strengthens vessel walls by regulating endothelial cell quiescence, stability of cell-to-cell contacts, and differentiation of mural cells. Transforming growth factor-β and PDGF-B gene expression are induced by hypoxia through the HIF-1 transcriptional pathway commonly utilized by hypoxia-sensitive genes (Ratcliff et al., 1998).

Tumor necrosis factor-α promotes endothelial cell tube formation in vitro and inhibits endothelial cell proliferation (Papetti and Herman, 2002). Tumor necrosis factor-α and fibroblast growth factors (**FGF**) stimulate angiogenesis in chick chorioallantoic membrane

(**CAM**) assays and cornea pocket models. In fact, many studies have demonstrated profound angiogenic influences of FGF in these and other endothelial cell assays, but the physiological relevance of these assays is questionable (Yancopoulos et al., 2000). There is limited ability to evaluate the functionality of vessels induced in CAM and cornea pocket assays (Yancopoulos et al., 2000). Furthermore, FGF knockout mice have no phenotype (Papetti and Herman, 2002; Yancopoulos et al., 2000). Regardless, FGF may play a role in angiogenesis during tissue repair (Papetti and Herman, 2002).

Leptin Angiogenic Studies

Recent studies have addressed the possibility that leptin may either induce angiogenesis or influence angiogenic factors (Bouloumie et al., 1998; Sierra-Honigmann et al., 1998; Cao et al., 2001; Oda et al., 2001; Park et al., 2001; Artwohl et al., 2002; Goetze et al., 2002). Long-form leptin receptor is expressed by endothelial cells in vitro and in human and mouse adipose tissue but is not expressed by adipose tissue endothelial cells in leptin receptor-deficient (db/db) mice (Bouloumie et al., 1998; Sierra-Honigmann et al., 1998; Bornstein et al., 2000). Leptin increases tyrosine phosphorylation of several endothelial cell proteins in vitro, including Erk1/2 and STAT3, indicating the functionality of the endothelial cell leptin receptor (Bouloumie et al., 1998; Sierra-Honigmann et al., 1998).

Leptin studies have included cornea pocket and CAM assays and cultures of several types of endothelial cells, including human umbilical vascular endothelial cells (**HUVEC**) and porcine aortic endothelial cells (Bouloumie et al., 1998; Sierra-Honigmann et al., 1998; Park et al., 2001; Cao et al., 2001; Artwold et al., 2002; Goetze et al., 2002). Leptin induces angiogenesis in cornea pocket and CAM assays and influences endothelial cell migration, proliferation, survival, and apoptosis in vitro (Bouloumie et al., 1998; Sierra-Honigmann et al., 1998 ; Park et al., 2001; Cao et al., 2001; Artwold et al., 2002; Goetze et al., 2002). Furthermore, leptin increases smooth muscle cell (**SMC**) proliferation and migration (Oda et al., 2001) and SMC and endothelial cell expression of MMP proteinases and associated proteins (Park et al., 2001). And leptin increases mouse adipose tissue Ang 2 and VEGF expression (Cohen et al., 2001).

The proposed angiogenic nature of leptin may be tentative for several reasons, including the absence of gene knockout or overexpression models to validate the current studies. And, when leptin and VEGF were directly compared, higher leptin levels were often necessary to match the VEGF effect. Furthermore, cornea pocket assays were widely used in leptin studies but may have limited value (Yancopoulos et al., 2000). The physiological relevance of cornea pocket models and CAM assays has been questioned because there is limited ability to evaluate the functionality of the induced vessels (Yancopoulos et al., 2000). Also, many growth factors/cytokines exhibit angiogenic properties, such as TGF-α, EGF, CSF, and interferon-γ, when tested in these assays.

Despite the potential limitations of assays used in leptin studies, there are several notable results regarding leptin's influence on adipogenesis. For instance, leptin induced capillaries in cornea pockets from normal rats but failed to do so in cornea pockets from fa/fa rats (Sierra-Honigmann et al., 1998). Leptin and VEGF induced similar vascular fenestrations in newly formed cornea pocket vessels and similarly increased vascular permeability overall in mice (Cao et al., 2001). And leptin and VEGF similarly influenced endothelial cell viability (Bouloumie et al., 1998). Furthermore, leptin may potentiate VEGF-mediated angiogenesis because leptin increased endothelial cell VEGF secretion in a dose-dependent manner (Park et al., 2001) and acted synergistically with VEGF- and FGF-driven angiogenesis in cornea pocket assays (Cao et al., 2001). Therefore, leptin may indirectly influence some aspects of angiogenesis by potentiating VEGF secretion and uptake.

Adipose tissue leptin and VEGF expression are similarly regulated by insulin and hypoxia. Leptin and VEGF gene expression are induced by hypoxia through the hypoxia-induced factor (**HIF**)-1 transcriptional pathway (Yancopoulos et al., 2000; Grosfeld et al., 2002; Ambrosini et al., 2002). Studies of human preadipocytes (PAZ6 cells) and isolated adipocytes showed that hypoxia or chemical inducers of cellular hypoxia ($CoCI_2$) induced leptin gene expression, leptin promoter activity (PAZ6 cells), and leptin secretion in conditioned media, whereas hypoxia decreased expression of several adipocyte marker genes in PAZ6 cells (Grosfeld et al., 2002). Hypoxia-induced factor-1 transcriptional activation also mediates hypoxia-induced leptin gene expression in human fibroblasts (Ambrosini et al., 2002), which may play a role in vascular remodeling (Stenmark et al., 2002). Hypoxia-induced factor-1 activation of leptin gene expression is very intriguing because a general system of gene regulation by oxygen involves fewer than 25 HIF-1 transcriptionally activated genes (Ratcliffe et al., 1998; Wenger, 2000).

Evidence That Vasculogenesis or Angiogenesis Regulates Adipogenesis

Several diverse lines of evidence indicate that blood vessels or blood vessel development may influence adipocytes or adipogenesis (Table 2). During fetal development, arteriolar differentiation precedes adipocyte development and differentiation of blood vessel extracellular matrix (ECM) precedes differentiation of adipocyte ECM (Crandall et al., 1996; Table 2). Possibly, differentiated blood vessels may induce or enhance preadipocyte spreading and migration during adipogenesis (Hausman et al., 1996). During postnatal development, VEGF expression and resulting angiogenesis may augment or precipitate adipogenesis in white and brown adipose tissue (Table 2). And, studies of knockout

Table 2. Evidence that angiogenic factors or stages and/or components of angiogenesis may regulate adipocyte development or adipose tissue accretion[a]

Factor/component/approach	Regulation/outcome	Reference
VEGF	Cold-induced expression in BAT maximized in 1 h	Asano et al. (1997, 1999)
VEGF	Increased expression associated with increased angiogenesis during rebound weight gain after diet restriction	Morimura et al. (2001)
Adipose tissue ontogeny	Differentiation of vasculature precedes adipocyte differentiation	Hausman and Thomas (1984, 1985); Hausman (1987); Hausman et al. (1991)
Plg-deficient mice	Fat tissue weight, body weight, and fat tissue blood vessels decreased	Hoover-Plow et al. (2002)
t-PA-deficient mice—high-fat fed	Fat tissue weight, body weight, fat cell size, and endothelial cells increased	Morange et al. (2002)
Antiangiogenic factor treatment in vivo	Decreased fat tissue weights	Rupnick et al. (2002)

[a]VEGF = vascular endothelial growth factor; Plg = plasminogen; t-PA = tissue plasminogen activator; BAT = brown adipose tissue.

mice indicate that extracellular proteolysis may regulate adipose tissue angiogenesis and adipocyte development (Table 2). Finally, treatment of mice with antiangiogenic factors decreased fat pad weights by 12 to 22% and decreased body weights in a dose-dependent and reversible manner (Rupnick et al., 2002; Table 2). Further studies are necessary to establish the nature of the influence of antiangiogenic factors on adipose tissue development.

Implications

The relationship between angiogenesis and adipogenesis in meat animals has been examined morphologically and cytochemically, but the expression of major regulators of angiogenesis by meat animal adipose tissue has not been examined. In particular, it is critical that the relationship between vascular endothelial growth factor expression and secretion and brown adipose tissue and white adipose tissue adipogenesis be examined in meat animals. Furthermore, the role of vascular endothelial growth factor in dictating depot-dependent adipose tissue traits warrants study in meat animals. Preliminary and indirect evidence indicate that developing blood vessels in fat tissue represent a potential target for regulating fat cell development in the growing meat animal.

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